Docket No. 13720-105089

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	CIFRA et al.		
		Group Art Unit:	1614
Serial No.:	10/692,191		
		Examiner:	Leslie ROYDS
Filed:	October 22, 2003		
		Confirmation No.	8424

For: MODULATION OF ZINC LEVELS TO IMPROVE TISSUE PROPERTIES

VIA EFS

DECLARATION OF DR. JACOB M. WAUGH

UNDER 37 C.F.R. §1.132

I, Jacob M. Waugh, hereby declare the following:

1. I graduated from Rice University with a B.A. in Chemistry and Biochemistry. I received my M.D. from Baylor College of Medicine. I was a Senior Scientist at Stanford University School of Medicine from 2001 to 2004, where I worked on tissue engineering applications. I have authored over 40 research manuscripts and publications. Currently, I am the Chief Scientific Officer of Revance Therapeutics, Inc.

2. I am a named inventor on U.S. Application No. 10/692,191 ("the '191 application), titled "Modulation of Zinc Levels to Improve Tissue Properties".

3. The presently claimed invention in the '191 application generally relates to methods for increasing the elastin content in the skin of a subject by topically applying a zinc-containing compound in the area to be treated. Elastin is a protein that is naturally found

in skin and is responsible for the skin's elasticity. Humans naturally produce elastin, although the rate of production decreases with age, leading to the gradual appearance of wrinkles and sagging skin. When a zinc-containing compound is applied in accordance with the invention, it causes a net increase in the elastin content in the treated area of the skin, thereby ameliorating the physiological symptoms associated with low elastin levels. In this way, the claimed invention provides for a method of treating wrinkles and sagging skin.

4. In addition to synthesizing elastin, the human body produces elastase, an enzyme that is capable of breaking down elastin. When the activity of the elastase in a particular region of the skin is high, it causes a net loss of elastin, a condition known as elastosis. The ability of elastase to break down elastin in a certain area of the skin can be affected by the local concentration of zinc in that area.

5. I have read the Office Action issued by the U.S. Patent and Trademark Office ("USPTO") on March 18, 2008 for the '191 application. I have also read the two references that were cited against the pending claims of this application: (1) U.S. Patent 6,573,299 to Petrus ("Petrus"); and (2) an abstract corresponding to an article by Uitto entitled "Connective Tissue Biochemistry of the Aging Dermis. Age-Related Alterations in Collagen and Elastin" Dermatol Clin. 1986 Jul; (4(3):433-436.

6. Based on my review of the Office Action and cited references, I respectfully disagree with the Examiner's conclusion that the pending claims are obvious over the cited

references. As discussed below, my conclusions are based on the following observations: (1) none of the cited references recognize that application of zinc compounds at high concentrations can lead to irritation or sloughing; (2) none of the cited references recognize that application of zinc compounds at high concentrations can lead to elastosis, which is counterproductive to the treatment of wrinkles or sagging skin; and (3) none of the cited references show that topical administration of zinc compositions with zinc in the claimed concentration ranges can lead to the production of elastin without irritation, sloughing, or elastosis.

7. I have performed experiments to demonstrate the effect of zinc on the elastin production in murine skin. The zinc-containing formulations that were used in this study had zinc concentrations of 1 μM, 1 mM, 10 mM, and 1 M and were applied topically. In addition, a control experiment was performed in which the formulation contained phosphate buffered saline (PBS), but no zinc. The zinc source used for this study was zinc citrate, although any zinc compound or salt that is capable of delivering free Zn ions and that is non-toxic in the claimed concentration ranges may be used as a zinc source.

8. The zinc formulations were topically applied using a method similar to the method described in Example 1 of the '191 application. Briefly, C57BL6 mice at about 6-8 weeks of age were anesthetized with 3% isoflurane by inhalation and were shaved at the mid-scapular dorsal region. The zinc formulations were applied at 0.2 cc per day for a 21 period (N=4 per test group). After 21 days of application, the treated skin segment was harvested and fixed in 10% neutral buffered formalin, then rinsed in 70% ethanol and

Compared to the images in Figures 2-4, the image in Figure 5 shows a marked decrease in the number and density of the dark, thread-like features in the dermis associated with elastin. Instead, the image shows black "chunky" features characteristic of elastin fibers that have been broken down by elastase. Moreover, the image shows an increased number of neutrophils, which secrete elastase and are associated with inflammation. This decrease in the amount of elastin in the dermis can be attributed to an increase in elastase activity caused by the higher zinc concentration.

10. To roughly quantify the amount of elastin produced by application of these various zinc citrate formulations, I performed a computerized image analysis on the photomicrographs shown in Figures 1-5. The computerized image analysis involved comparing the number of black pixels in each photomicrograph with the total number of pixels. The analysis is based on the assumption that the number of black pixels is proportional to the amount of elastin present, since elastin appears in photomicrographs as black thread-like features following Verhoeff elastica staining. It should be noted that other features corresponding to neutrophils also appear black, so that the computer image analysis tends to overestimate the amount of elastin. The results of the computerized analysis is shown in the following table:

Table 1: Results of Computerized Image Analysis of Elastin Production in a Murine Skin Model

	sum of black pixels	Total pixels	% black pixels
PBS	10236	543926	1.881874
1μm	32345	791298	4.087588
1mM	23871	484570	4.926223
10mM	36323	245691	14.78402
1M	21091	523212	4.031062

11. The results shown in Table 1 indicate that the amount of elastin produced increases as the concentration of the zinc in the topical formulation increases from 1 μM to 10 mM. With respect to the 1 M zinc formulation, the relative number of black pixels in the image appears to be comparable to the relative number of black pixels in the image corresponding to the 1 μM formulation. However, as noted above, a visual inspection of Figure 5 indicates that there are very few dark, continuous thread-like features associated with elastin. Accordingly, I attribute the relatively high number of black pixels in Figure 5 (the image corresponding to the 1 M formulation) to the increased presence of degraded elastin fibers and neutrophils, which also appear to be black after Verhoeff elastica staining.

12. Based on these and other studies that I have performed, I have determined that topical application of zinc-containing compositions having a zinc concentration greater than about 100 mM at the dosing rate described above can lead to local inflammation and/or sloughing. Moreover, topical application of zinc-containing compositions have a zinc concentration greater than 1 M at the dosing rate described above can lead to a net loss of

embedded in paraffin. The paraffin-embedded specimens were sectioned at 4-6 microns and stained with Verhoeff elastica stain for morphological assessment of elastin content. High resolution digital micrographs were taken using a Diagnostic Instruments SPOT camera (as displayed on a Nikon E600 epifluorescence microscope with plan-apochromat lenses). Images were analyzed using Image Pro Plus Software (Media Cybernetics, Silver Spring, MD) to determine total cross sectional area of epidermis over standardized lengths.

9. Figures 1-5 which accompany this declaration show photomicrographs of murine skin prepared in the manner described above. Figure 1 corresponds to the control experiment, in which no zinc was topically applied to the skin of the test animal. The thick, dark layer near the top of the micrograph is the epidermis of the animal. Elastin, which appears as black, continuous, thread-like features after Verhoeff elastica staining, is seen to be generally present throughout the underlying dermis. As the topical application of PBS is not expected to cause an increase in elastin production, the elastin shown in Figure 1 corresponds to the elastin that was naturally present in the dermis of the test animal. Figures 2, 3, and 4 show photomicrographs corresponding to topical application of 1 μM, 1 mM, and 10 mM formulations, respectively. All of these figures show a marked increase in the number and density of the dark, continuous, thread-like features in the dermis, indicating an increase in the amount of elastin, as compared to the control experiment. This result indicates that the application of the 1 μM, 1 mM, and 10 mM formulations leads to an increase in the amount of elastin in the dermis. Figure 5 shows a photomicrograph taken after topical application of the 1 M zinc citrate formulation.

elastin (i.e., elastosis). Neither Petrus nor Uitto recognize or report these zinc-concentration dependent phenomema.

13. I am familiar the anti-wrinkle formulation known as Relastin Eye Silk, having been a member of the team that developed this product.

14. Relastin Eye Silk contains cyclopentasiloxane, dimethicone crosspolymer, dimethicone, phenoxyethanol, zinc complex, mica, and titanium dioxide. These ingredients are listed on the packaging of the Relastin Eye Silk product.

15. Cyclopentasiloxane, dimethicone crosspolymer, and dimethicone are organosilicon polymers that are useful for providing the proper consistency for topical cream products, such as Relastin Eye Silk. Phenoxyethanol is a well known cosmetic preservative. Mica and titanium dioxide are minerals that improve the luster, color, and appearance of the Relastin Eye Silk cream, both prior to and after application. However, none of these ingredients are expected to promote the production of elastin in skin upon topical application.

16. The "zinc complex" in Relastin Eye Silk is one of the zinc-containing substances recited in Applicants' claims. Moreover, the concentration of zinc in Relastin Eye Silk falls within the claimed concentration ranges in the '191 application.

17. Based on my laboratory studies, as well as my understanding of the chemical properties of the components of Relastin Eye Silk, I conclude that the ability of Relastin Eye Silk to promote the production of elastin and to treat wrinkles results from the presence of the zinc complex in the formulation.

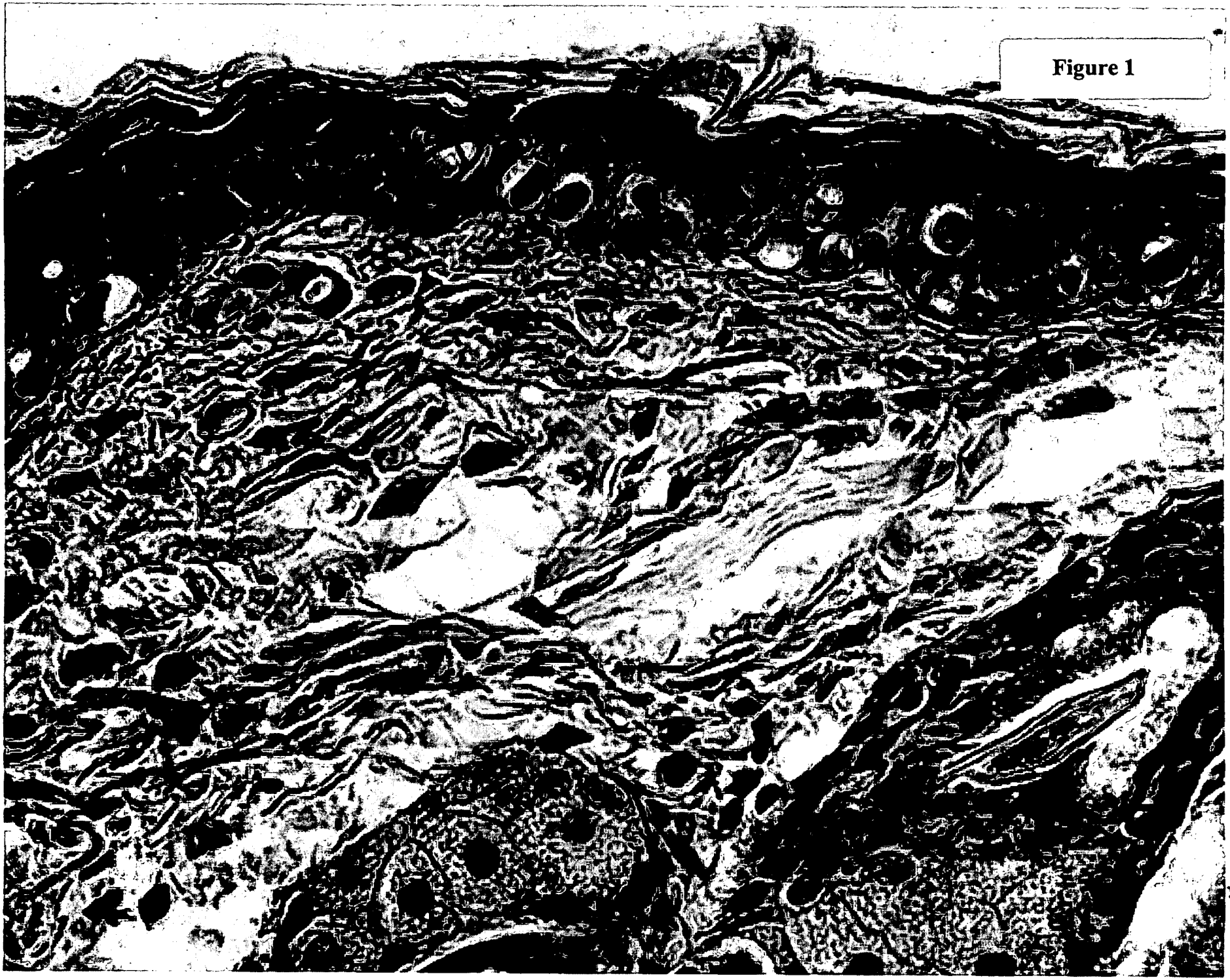
18. All of the statements made of my own knowledge are true and all of the statements made on information and belief are believed to be true. I hereby acknowledge that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of this application or any patent issuing thereon.

Date 15APR09

Signed _____

Jacob M. Waugh

Figure 1



PB

Figure 2

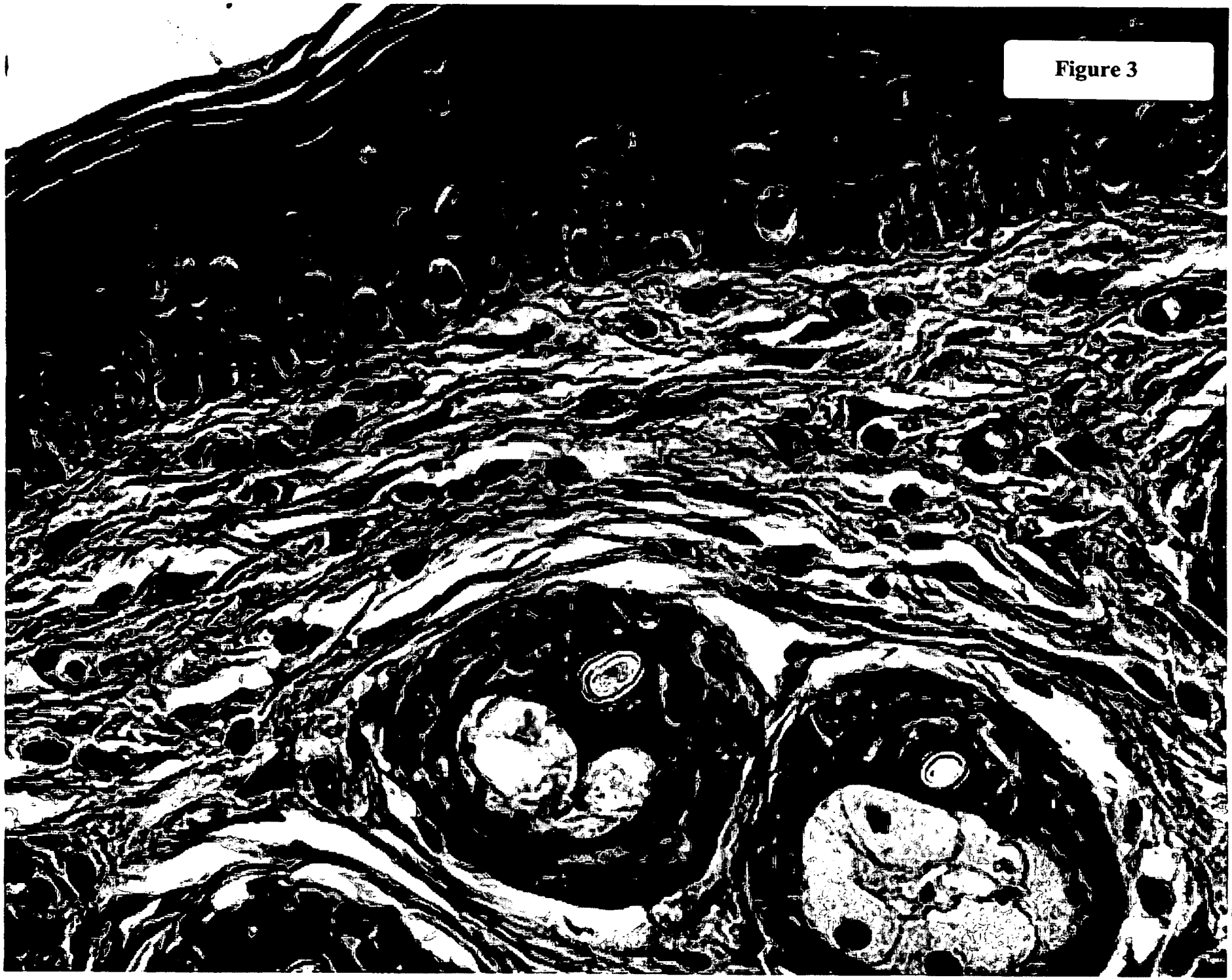


Figure 3

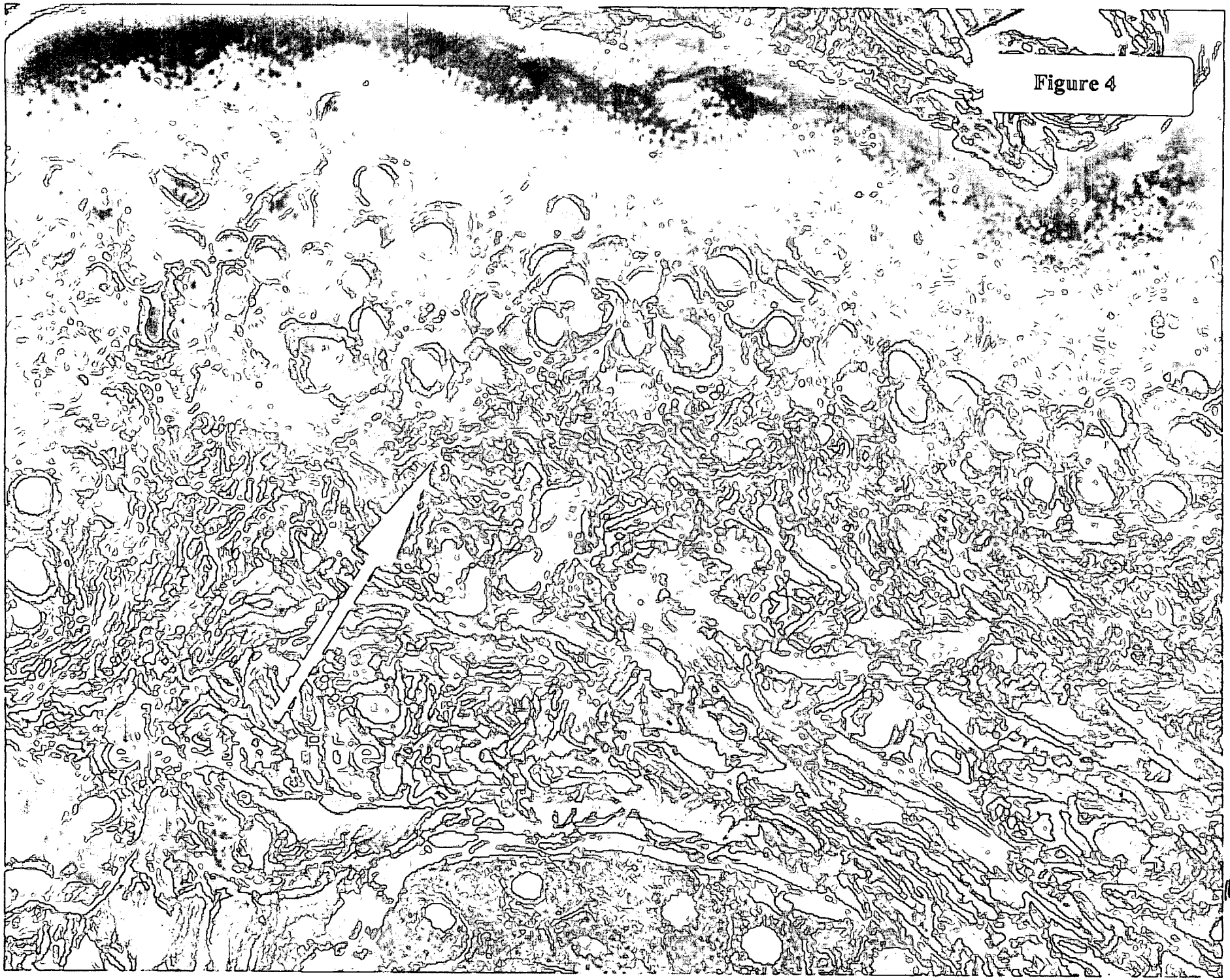


Figure 4

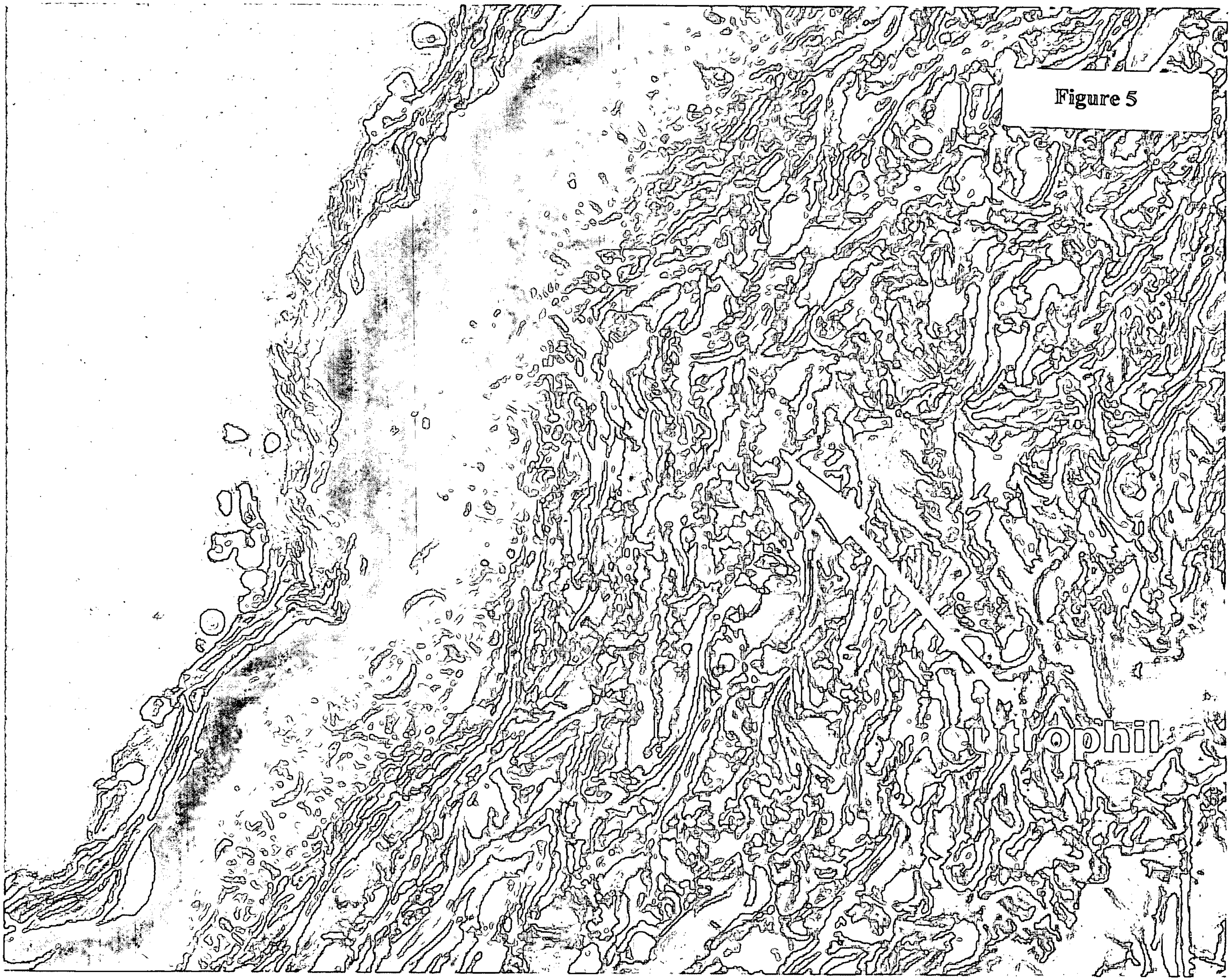


Figure 5